Replication in Eukaryotic organism

→ DNA is linear

→ multiple origin int [in Yeast ≈ 1000 origin; in Human ≈ 10,000 origin]

→ Single Round of Replication

→ Replication Speed = 50 - 100 nt/sec

- slower than prokaryote

Reason - Histone protein int DNA int in Condensed form

→ Replication is Bidirectional

→ Human Genome Size : 3×10^9 bp

$$\text{Replication duration} = \frac{3 \times 10^9}{100 \times 1000 \times 2 \times 60}$$

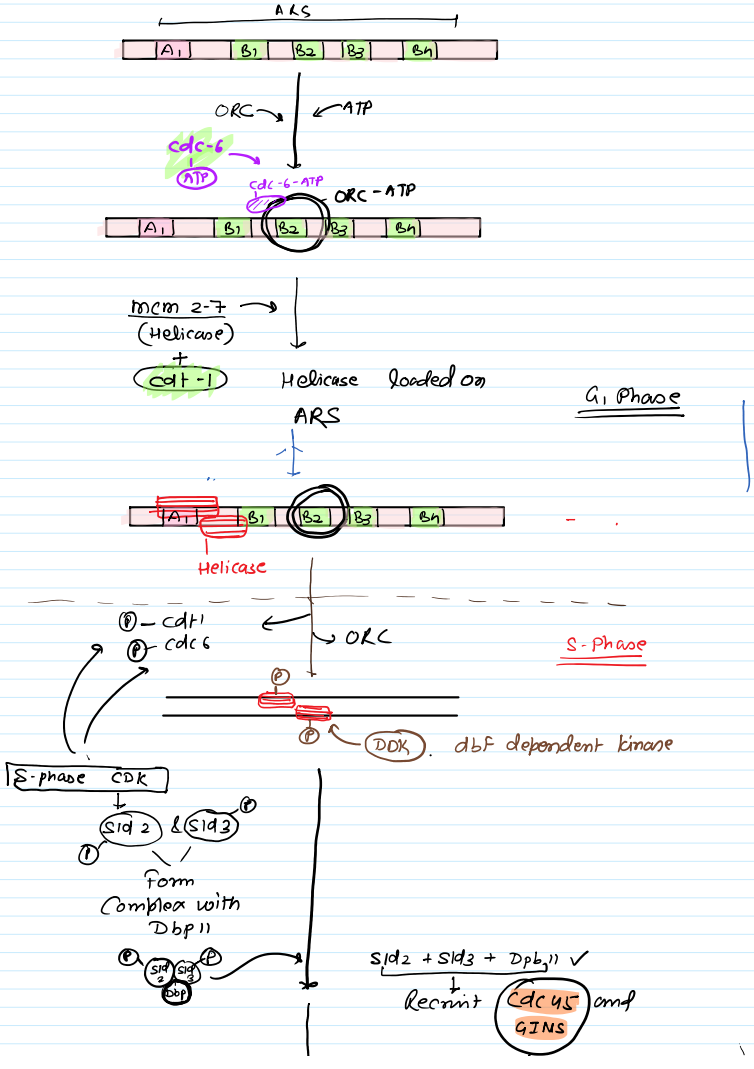
if No. of origin = 1000

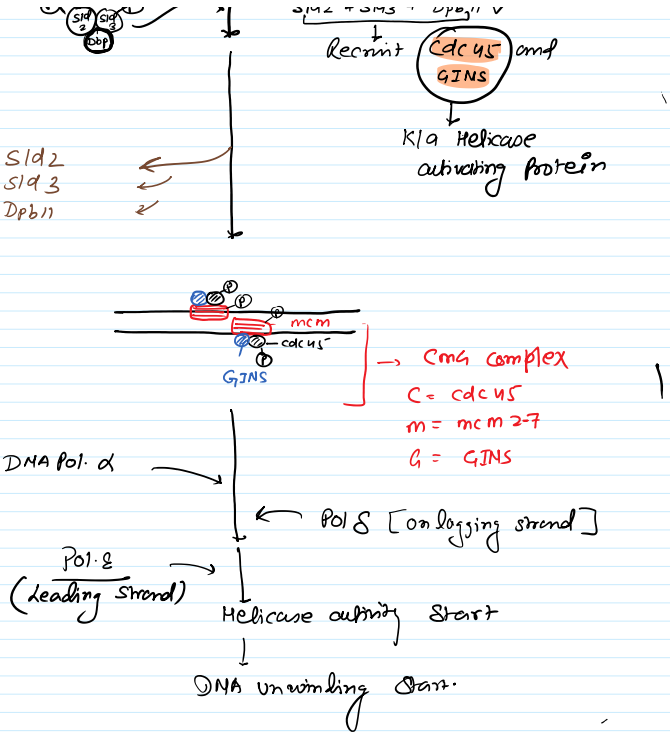
$$= \frac{3000}{12} = 250 \text{ min}$$

$$\frac{250 \text{ min}}{60} = 4.1 \text{ hr}$$

Replication duration = 4.1 hr = S phase duration

Origin of Replication in Yeast



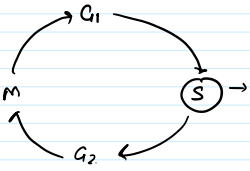


Helicase loading occurs in G_1 phase

Cmg Complex formation occurs in S- phase

Pre-Replication Complex [mcm + licencing factor]

cdt-1
cdc-6



→ Cmg complex formation
- Replication start
- licencing factor Phosphorylated

cdc-6 (P) → degrade
cdt-1 (P) → inactive

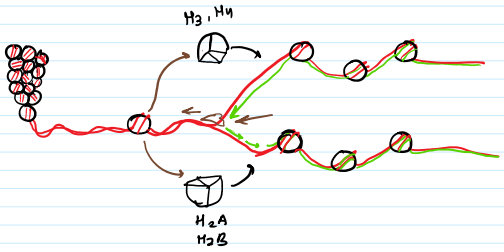
- licencing factor is required for Helicase loading
- licencing factor inactivated once Replication is initiated
- licencing factor for Next Replication Cycle, formed in G_1 phase

↓

Replication initiation - occurs once in a cell cycle.

Histone Synthesis

↳ Semiconservative.

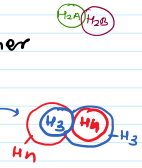


Chromatin Assembly Factor [CAF] → are chaperons

↓

Facilitate Assembly of Nucleosome

Chaperons
- NAP-1 → Transfer H_2A & H_2B Heterodimer on New DNA
- CAF-1 → Transfer H_3 & H_4 Tetramer on New DNA



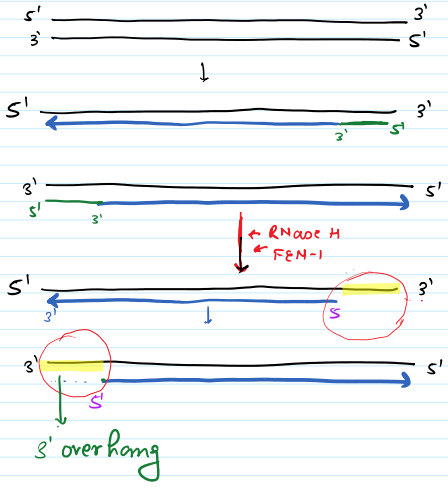
- Some modification can be seen in H_3 Histone

eg Acetylation

- Acetylated Histone → Euchromatin
  - Transcriptionally active
- Non-Acetylated Histone – Heterochromatin
  - ↓
  - Transcriptionally inactive

In Eukaryote – End Replication Problem

Due to linear DNA



3' overhang is observed on telomeric End of Chromosome

↓

Telomeric End contain tandom Repeats & does not Contain Functional gene

↓

After each Replication Cycle Telomere Shortening can be seen

↓

after 35-40 division of Somatic cell Stop dividing because now gene start loosing at the End due to Shortening

↓

Considered as DNA damage

↓

Cell cycle arrest

↓

Kia Hay Hick Limit

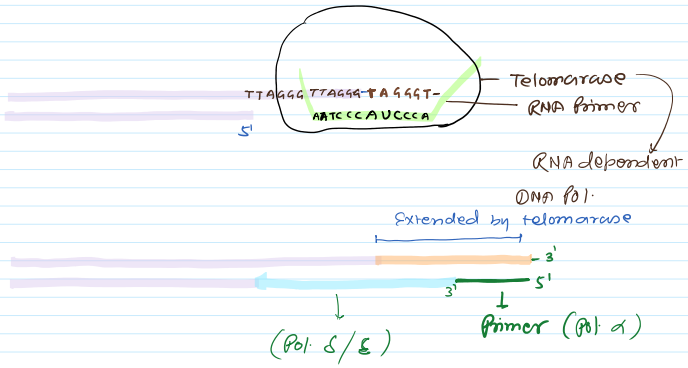
Cell cycle arrest

↓

Kla Hay Flick Limit

In Transformed cell [Cancerous cell] and Stem cell → Contain Telomarase ↓ maintain Telomeric length

#



If Telomeric end (Elongated)

↓

Binds → Rap-1 + Rif.1 + Rif 2 Protein complex (in yeast)

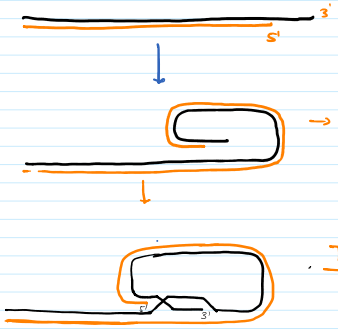
↓

Telomarase activity

In human → instead of Rap 1 & Rif complex

POT-1 + TIN2 + Rap 1 + TRF 1 & 2

complex is tnf



Telomere – GT Rich Sequence (minisatellite DNA)

eg - Human telomere Seq = 5'-TTAGGG-3'

T_2 A_1 G_3

telomere is protected by Shelterin protein

↓

Rap-1 + TIN-2 + TPP-1 POT-1

In Drosophila - Telomeric Seq. - A_0 T_2 G_4

(TT GGGG)

Replication of Extra Chromosomal DNA

(Plasmid) → +nt in Bacteria & Eukaryote

→ Plasmid is not a basic Requirement or Absolute Requirement

- Plasmid Replication – Energy investment
  - Antibiotic Resistance
  - Heavy metal "

Plasmid – Closed Circular DNA

Small Sized

- 10 - 100 kb
- Relaxed Plasmid
- ↑ Copy No.
- 10 - 40 copies +nt in a cell
  - Sometimes 200 copies +nt

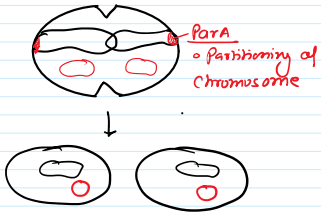
eg- Non-Conjugative Plasmid
eg. Col. E1 Plasmid

Large Sized

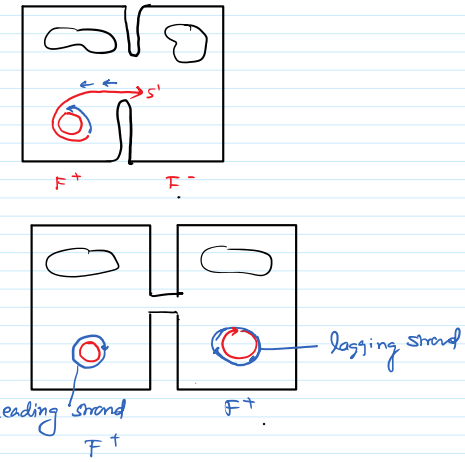
- 100 - 300 kbp
- Kia Stringent Plasmid [Supercoiled]
- Low Copy No. Plasmid
- 1 - 23 Copies +nt in a cell
- Conjugative Plasmid
  eg- P. Plasmid

- Transfer of Bacterial Plasmid from 1 cell to another

(i) Partition System



(ii) cell division delay system



* Conjugative Plasmid transfer during Conjugation

→ Sex pilli of Bacteria helps in Conjugation

↓

Rolling circle mode of Replication
(as mentioned in cell division delay system)

→ Nick Required to start Replication

→ 5' End of Nicked Strand is Transfered into New cell (F^-)

→ lagging strand Synthesis start on Transfered strand

→ F^+ cell Plasmid

OH promotered strand

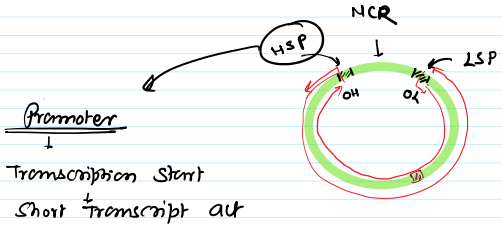
↳ F^+ cell Plasmid

↳ Leading Strand Synthesis

Mitochondrial DNA Replication

mitochondrial genome Kia Chondriome

↳ Closed circular Ds DNA



HSP = Heavy Strand Promoter

LSP = Light Strand Promoter

NCR = Non-Coding Region

Replication Required -

- Helicase = Kia Twinkle
- DNA Pol. γ -
  - ↳ Trimeric Protein
    - DNA Pol. γ B (2) → Holds Template & Increase Processivity
    - " Pol. γA (1) ↳ Catalytic Subunit
      - 5' → 3' polymerase
      - 3' → 5' Exonuclease

Mechanism

- Replication is starts in unidirectional manner from OH [specific origin for Heavy Strand]
  - ↓
  - init in HSP

* when 1 Strand that start from OH is synthesized 2/3rd then 2nd Strand Synthesis begins from OL Seq.
  - ↓
  - init in LSP

* Mitochondrial DNA Replication Termination is Associated with
  - ↓
  - Termination Associated Seq. (TAS)

DNA Damage